

In the Claims

We claim:

Claim 1 (Currently amended): A method of sequencing a target polynucleotide comprising ~~the steps of:~~

- (a) ~~Carrying carrying~~ out template derived nucleotide synthesis ~~utilising a labelled~~ utilizing a labeled nucleotide;
- (b) detecting the presence or absence of said ~~labelled~~ labeled nucleotide;
- (c) replacing said ~~labelled~~ labeled nucleotide with an ~~unlabelled~~ unlabeled nucleotide; and
- (d) repeating steps a) to c) with the proviso that if said ~~labelled~~ labeled nucleotide is ~~labelled~~ labeled with a label directly attached to the nucleotide, then the replacement of said ~~labelled~~ labeled nucleotide comprises removal of the whole of said ~~labelled~~ labeled nucleotide and replacement with an ~~unlabelled~~ unlabeled nucleotide, and only said ~~labelled~~ labeled nucleotide can be removed.

Claim 2 (Currently amended): ~~A method as claimed in claim 1~~ The method of claim 1, wherein said target polynucleotide is attached to a solid surface.

Claim 3 (Currently amended): ~~A method as claimed in claim 1 or claim 2~~ The method of claim 1, wherein said ~~labelled~~ labeled nucleotide is ~~labelled~~ labeled with a fluorescent tag.

Claim 4 (Currently amended): ~~A method as claimed in claim 3~~ The method of claim 3, wherein said fluorescent tag is attached directly to said nucleotide.

Claim 5 (Currently amended): ~~A method as claimed in claim 3 or claim 4~~ The method of claim 3, wherein said ~~labelled~~ labeled nucleotide is attached to a quencher at the gamma position, and said fluorescent tag is attached at the 3' position or to the base.

Claim 6 (Currently amended): ~~A method as claimed in claim 4 or claim 5~~ The method of claim 4, wherein step (c) comprises chemically inactivating or photobleaching said fluorescent tag.

Claim 7 (Currently amended): ~~A method as claimed in claim 4~~ The method of claim 4, wherein ~~step (c)~~ comprises removal of said ~~labelled~~ labeled nucleotide and replacement with an ~~unlabelled~~ unlabeled nucleotide, wherein said ~~unlabelled~~ unlabeled nucleotide is a degradation resistant nucleotide.

Claim 8 (Currently amended): ~~A method as claimed in any one of claims 1 to 4~~ The method of claim 1, wherein said ~~labelled~~ labeled nucleotide is a degradation labile nucleotide.

Claim 9 (Currently amended): ~~A method as claimed in anyone of claims 1 to 3~~ The method of claim 1, wherein said ~~labelled~~ labeled nucleotide is ~~labelled~~ labeled with a nanoparticle.

Claim 10 (Currently amended): ~~A method as claimed in claim 9~~ The method of claim 9, wherein said nanoparticle is a semiconductor nanocrystal.

Claim 11 (Currently amended): ~~A method as claimed in any one of claims 3, 9 or 10~~ The method of claim 3, wherein said fluorescent tag or said nanoparticle are attached to said ~~labelled~~ labeled nucleotide by a linkage.

Claim 12 (Currently amended): ~~A method as claimed in claim 11~~ The method of claim 11, wherein said linkage comprises a binding pair.

Claim 13 (Currently amended): ~~A method as claimed in claim 12~~ The method of claim 12, wherein said binding pair comprises streptavidin and biotin or an analog thereof.

Claim 14 (Currently amended): ~~A method as claimed in claim 13~~ The method of claim 13, wherein said biotin or analogue thereof is 2-Iminobiotin or Desthiobiotin.

Claim 15 (Currently amended): ~~A method as claimed in claim 12 or claim 13~~ The method of claim 12, wherein said fluorescent tag or nanoparticle is conjugated to said streptavidin.

Claim 16 (Currently amended): ~~A method as claimed in any one of claims 11 to 13~~ The method of claim 13, wherein said linkage comprises a cleavable bond.

Claim 17 (Currently amended): ~~A method as claimed in any one of claims 12 to 16~~ The method of claim 12, wherein step (b) comprises ~~incorporation~~ incorporating of an ~~unlabelled~~ unlabeled nucleotide adapted for the attachment of a fluorescent tag or nanoparticle; and attaching said fluorescent tag or nanoparticle to said ~~unlabelled~~ unlabeled nucleotide.

Claim 18 (Currently amended): ~~A method as claimed in any one of claims 9 to 17~~ The method of claim 9, wherein step (c) comprises removing the fluorescent tag or nanoparticle from said ~~labelled~~ labeled nucleotide.

Claim 19 (Currently amended): ~~A method as claimed in claim 18~~ The method of claim 18, wherein said ~~fluorescent~~ fluorescent tag or ~~said~~ nanoparticle is removed from said ~~labelled~~ labeled nucleotide by cleaving the cleavable bond in the linkage attaching said fluorescent tag or ~~said~~ nanoparticle to the nucleotide.

Claim 20 (Currently amended): ~~A method as claimed in claim 15~~ The method of claim 15, wherein said linkage ~~attaches~~ attaches one member of a binding pair to the nucleotide, and the other member of the binding pair is attached to said nanoparticle.

Claim 21 (Currently amended): ~~A method as claimed in claim 9~~ The method of claim 9, wherein said linkage comprises a binding member attached by a cleavable bond to said nucleotide and the other binding member is attached to said fluorescent tag.

Claim 22 (Currently amended): ~~A method as claimed in claim 21~~ The method of claim 21, wherein step (d) comprises ~~removal of~~ removing said fluorescent tag by cleaving said cleavable bond.

Claim 23 (Currently amended): ~~A method as claimed in any one of claims 1 to 3~~ The method of claim 1, wherein step (b) is carried out by ~~means of~~ an imaging technique ~~utilising~~

~~FRET (fluorescent resonance energy transfer)~~ utilizing fluorescent resonance energy transfer (FRET).

Claim 24 (Currently amended): ~~A method as claimed in claim 23~~ The method of claim 23, wherein said target polynucleotide is treated with a DNA stain.

Claim 25 (Currently amended): ~~A method as claimed in claim 23 or claim 24~~ The method of claim 23, wherein said ~~labelled~~ labeled nucleotide is ~~labelled~~ labeled with a label that acts as a FRET partner.

Claim 26 (Currently amended): ~~A method as claimed in anyone of claims 23 to 25~~ The method of claim 23, wherein ~~steps (a)-(c)~~ occur simultaneously.

Claim 27 (Currently amended): ~~A method as claimed in any one of claims 1 to 3~~ The method of claim 1, wherein said ~~labelled~~ labeled nucleotide is an oligonucleotide and ~~step (a)~~ comprises ligating said oligonucleotide to a primer annealed to said target polynucleotide.

Claim 28 (Currently amended): ~~A method as claimed in claim 27~~ The method of claim 27, wherein ~~step (c)~~ comprises contacting said oligonucleotide with a degradation agent to remove the label.

Claim 29 (Currently amended): ~~A method as claimed in claim 27 or claim 28~~ The method of claim 27, wherein said ~~ligation~~ ligating forms a degradation resistant bond.

Claim 30 (Currently amended): ~~A method as claimed in any one of claims 27 to 29~~ The method of claim 27, wherein said oligonucleotide comprises a degradation labile intranucleoside bond and ~~step (c)~~ comprises contacting said oligonucleotide with an agent that degrades said degradation labile intranucleoside bond.

Claim 31 (Currently amended): ~~A method as claimed in claim 30~~ The method of claim 30, wherein said degradation labile intranucleoside bond is between the terminal nucleotide which is ligated to said primer and the adjacent nucleotide.

Claim 32 (Currently amended): ~~A method as claimed in any one of claims 27 to 29~~ The method of claim 27, wherein the terminal nucleotide which is ligated to the primer is a deoxynucleotide, and at least the adjacent nucleotide is a ribonucleotide.

Claim 33 (Currently amended): ~~A method as claimed in claim 27~~ The method of claim 27, wherein said oligonucleotide comprises the structure:

Terminal nucleotide –N– nucleotide attached to a label –M– nucleotide attached to a ~~quencher~~ quencher,

wherein N and M are each independently a bond or at least one nucleotide; and M comprises a first degradation labile intranucleoside bond.

Claim 34 (Currently amended): ~~A method as claimed in claim 33~~ The method of claim 33, wherein said ~~ligation~~ ligating forms a degradation resistant bond.

Claim 35 (Currently amended): A method of sequencing a target polynucleotide comprising ~~the steps of~~:

(a) ~~Carrying out~~ carrying out template derived nucleotide synthesis by ligating ~~an labelled a~~ labeled oligonucleotide to a primer annealed to said target polynucleotide, wherein said ~~ligation~~ ligating forms a degradation resistant bond, and wherein said oligonucleotide comprises the structure:

Terminal nucleotide –N– nucleotide attached to a fluorescent label –M– nucleotide attached to a ~~quencher~~ quencher,

wherein N and M are each independently a bond or at least one nucleotide; and M comprises a first degradation labile intranucleoside bond;

- (b) ~~Contacting~~ contacting said oligonucleotide with a first degradation agent;
- (c) ~~Detecting~~ detecting the presence or absence of said labeled oligonucleotide;

(d) ~~Contacting~~ contacting said oligonucleotide with a second degradation agent; ~~and agent;~~
and

(e) ~~Repeating steps~~ repeating (a)-(d).

Claim 36 (Currently amended): ~~A method as claimed in claim 33 or claim 34~~ The method of claim 33, wherein N comprises a second degradation labile intranucleoside bond, wherein said second degradation labile intranucleoside bond is resistant to the ~~degradation~~ degradation agent used to degrade the first degradation labile intranucleoside bond.

Claim 37 (Cancelled)

Claim 38 (Currently amended): ~~A method as claimed in claim 27~~ The method of claim 27, wherein said oligonucleotide comprises the structure:

Terminal nucleotide –N– nucleotide attached to a fluorescent label –L– nucleotide attached to a ~~quencher~~ quencher,

wherein N is a bond or at least one nucleotide; and L comprises a number of nucleotides which together form a hairpin structure when said oligonucleotide is not annealed to said template.

Claim 39 (Currently amended): ~~A method as claimed in claim 38~~ The method of claim 38, wherein N comprises a degradation labile intranucleoside bond.

Claim 40 (Currently amended): ~~A method as claimed in claim 38~~ The method of claim 38, wherein said ~~ligation~~ ligating forms a degradation resistant bond.

Claim 41 (Currently amended): ~~A method as claimed in any one of claims 1 to 40~~ The method of claim 1, wherein said target polynucleotide forms part of an array.

Claim 42 (Currently amended): ~~A method as claimed in claim 39~~ The method of claim 39, wherein ~~step~~ (b) comprises measuring the signal generated by a plurality of said ~~labelled~~ labeled nucleotides.

Claim 43 (Currently amended): ~~A method as claimed in claim 39~~ The method of claim 39, wherein ~~step~~ (b) comprises detecting the presence or absence of said ~~labelled~~ labeled nucleotide for each individual polynucleotide.

Claim 44 (Currently amended): ~~A method as claimed in claim 43~~ The method of claim 43, wherein said ~~detection~~ detecting is carried out by ~~means of~~ single DNA molecule imaging.

Claim 45 (Currently amended): ~~A method as claimed in claim 44~~ The method of claim 44, wherein said single DNA molecule imaging technique is fluorescence resonance energy transfer (FRET).

Claim 46 (Currently amended): ~~A method as claimed in claim 45~~ The method of claim 45, wherein said polynucleotide is treated with a DNA stain.

Claim 47 (Currently amended): ~~A method as claimed in claim 46~~ The method of claim 46, wherein the label on said ~~labelled~~ labeled nucleotide acts as a fluorescence resonance energy transfer (FRET) ~~FRET~~-partner to said DNA ~~istain~~ stain.

Claim 48 (Currently amended): A method of comparing two or more polynucleotide sequences comprising:

- a) differentially ~~labelling~~ labeling the nucleotide sequences being compared;
- b) ~~immobilising~~ immobilizing said nucleotide sequences on a surface;
- c) detecting the locus of each nucleotide sequence; and
- d) sequencing said polynucleotide sequences using a method ~~as claimed in any of claims 1 to 47~~ comprising:

1) carrying out template derived nucleotide synthesis utilizing a labeled nucleotide;

(2) detecting the presence or absence of said labeled nucleotide;

(3) replacing said labeled nucleotide with an unlabeled nucleotide; and

(4) repeating 1) to 3) with the proviso that if said labeled nucleotide is labeled with a label directly attached to the nucleotide, then the replacement of said labeled

nucleotide comprises removal of the whole of said labeled nucleotide and replacement with an unlabeled nucleotide, and only said labeled nucleotide can be removed.

Claim 49 (Currently amended): ~~A method as claimed in claim 48~~ The method of claim 48, further comprising photobleaching the label prior to the sequencing of said polynucleotide sequence.

Claim 50 (Currently amended): A method of resolving ambiguities in a polynucleotide sequence comprising:

- a) identifying an area of ambiguity in a polynucleotide sequence;
- b) designing probes for each of the suspected sequence possibilities; and
- c) ~~utilising~~ utilizing the primers formed to sequence said polynucleotide sequence ~~utilising a method as claimed in any of claims 1 to 47~~ by a method comprising:

1) carrying out template derived nucleotide synthesis utilizing a labeled nucleotide;

(2) detecting the presence or absence of said labeled nucleotide;

(3) replacing said labeled nucleotide with an unlabeled nucleotide; and

(4) repeating 1) to 3) with the proviso that if said labeled nucleotide is labeled with a label directly attached to the nucleotide, then the replacement of said labeled nucleotide comprises removal of the whole of said labeled nucleotide and replacement with an unlabeled nucleotide, and only said labeled nucleotide can be removed.

Claim 51 (Currently amended): A method of sequencing mRNA comprising:

- a) contacting an array of probes designed to ~~hybridise~~ hybridize to mRNA molecules with a sample of mRNA under conditions whereby the mRNA will ~~hybridise~~ hybridize to said probes; and

- b) sequencing said mRNA ~~utilising~~ utilizing a method ~~as claimed in any one of claims 1 to 47~~

1) carrying out template derived nucleotide synthesis utilizing a labeled nucleotide;

(2) detecting the presence or absence of said labeled nucleotide;

(3) replacing said labeled nucleotide with an unlabeled nucleotide; and

(4) repeating 1) to 3) with the proviso that if said labeled nucleotide is labeled with a label directly attached to the nucleotide, then the replacement of said labeled nucleotide comprises removal of the whole of said labeled nucleotide and replacement with an unlabeled nucleotide, and only said labeled nucleotide can be removed.

Claim 52 (Currently amended): ~~A method as claimed in 51~~ The method of claim 51, wherein said probe is designed to ~~hybridise~~ hybridize to the polyadenylation signal, 5' cap, 3' tail or the poly A tail.

Claim 53 (Currently amended): A method of sequencing a target polynucleotide comprising ~~the steps of:~~

- (a) treating said target polynucleotide with an intercalating dye;
 - (b) extending a primer annealed to said target polynucleotide ~~utilising~~ utilizing a nucleotide ~~labelled~~ labeled with a label which acts as a fluorescence resonance energy transfer (FRET) ~~FRET~~ partner to said DNA intercalating dye;
 - (c) detecting the presence or absence of said nucleotide by means of an imaging technique that ~~utilises~~ FRET; and
 - (d) repeating ~~steps a-e~~ (a)-(c);
- wherein ~~steps~~ (a) and (b) can occur in any order.

Claim 54 (Currently amended): A method of sequencing a target polynucleotide comprising ~~the steps of:~~

- (a) extending a primer annealed to said target polynucleotide ~~utilising~~ utilizing a ~~labelled~~ labeled nucleotide wherein the label is directly attached to the nucleotide;
 - (b) detecting the presence or absence of said ~~labelled~~ labeled nucleotide within said extended primer;
 - (c) ~~removal of said labelled nucleotide~~ removing said labeled nucleotide, and ~~replacement of said labelled~~ replacing said labeled nucleotide with an ~~unlabelled~~ unlabeled degradation resistant nucleotide; and
 - (d) repeating ~~steps a-e~~ (a)-(c);
- wherein the 3' end of said primer comprises at least one degradation resistant nucleotide.

Claim 55 (Currently amended): A method of sequencing a target polynucleotide comprising ~~the steps~~:

- (a) extending a primer annealed to said target polynucleotide ~~utilising~~ utilizing a ~~labelled~~ labeled nucleotide wherein the label is attached to the nucleotide via a cleavable linkage;
- (b) detecting the presence or absence of said ~~labelled~~ labeled nucleotide within said extended primer;
- (c) cleaving said label from said nucleotide; and
- (d) repeating ~~steps a-e~~ (a)-(c).

Claim 56 (Currently amended): A method of sequencing a target polynucleotide comprising ~~the steps of~~ comprising:

- (a) extending a primer annealed to said target polynucleotide using a nucleotide attached by a cleavable linkage to one member of a binding pair;
- (b) contacting said nucleotide with a label attached to the other member of a binding pair under conditions such that the two members of the binding pair bind to one another;
- (c) detecting the presence or absence ~~or of~~ of said label;
- (d) ~~removal of~~ removing said label and said binding pair by cleaving said cleavable linkage; and
- (e) repeating ~~steps a-d~~ (a)-(d).

Claim 57 (Currently amended): A method of sequencing a target polynucleotide, comprising ~~the steps of~~:

- (a) carrying out template derived polynucleotide synthesis ~~utilising~~ utilizing a nucleotide ~~labelled~~ labeled with a fluorescence resonance energy transfer (FRET) ~~FRET~~ partner and at least one other ~~polymerisation~~ polymerization reaction component ~~labelled~~ labeled with a FRET partner;
- (b) determining the nucleotide incorporated by detecting FRET interactions; and
- (c) repeating ~~steps (a) and (b)~~.